Correspondence

Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease

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Defects in the *PKD1* and *PKD2* genes cause autosomal dominant polycystic kidney disease (PKD) in ~1 in 1000 adults worldwide. These genes encode polycystin-1 and polycystin-2, which are membrane proteins thought to be involved in a calcium signal transduction cascade that controls epithelial proliferation and differentiation. Individuals with mutations in these genes develop cysts in the ducts and tubules of their kidneys. These cysts lead to kidney failure in ~50% of affected individuals [1]. Similar to autosomal dominant PKD, autosomal recessive PKD also results in the formation of kidney cysts, but most human cases are caused by defects in a gene of unknown function [2].

How dominant and recessive PKD are related is unclear, but three recent findings suggest that both forms of the disease involve the non-motile primary cilia that extend from the kidney epithelial cells into the lumen of the collecting ducts and tubules. First, the *Caenorhabditis elegans* homologues of polycystin-1 and polycystin-2 are localized to the nematode's sensory cilia [3]. Second, the defective gene in the *Tg737^{orpk}* [4] recessive PKD mouse encodes a protein, IFT88, involved in intraflagellar transport and necessary for assembly of kidney primary cilia [5]. Finally, the defective gene in the *cpk* recessive PKD mouse encodes a novel ciliary protein [6]. Based on these findings, we hypothesize that the polycystins are located on primary cilia, and that the primary cilia have a sensory role in the polycystin-signaling pathway.

To determine if polycystin-2 is localized to the primary cilium, we examined its distribution in ciliated cultures of human and mouse kidney cells by immunofluorescence microscopy using the extensively characterized YCC2 anti-polycystin-2 antibody [7]. As reported, YCC2 detects a single band of ~110 kDa on western blots of protein extracted from mouse kidney and cultured human kidney cells (Figure 1A). Pretreating the antibody with recombinant polycystin-2 blocks this binding,

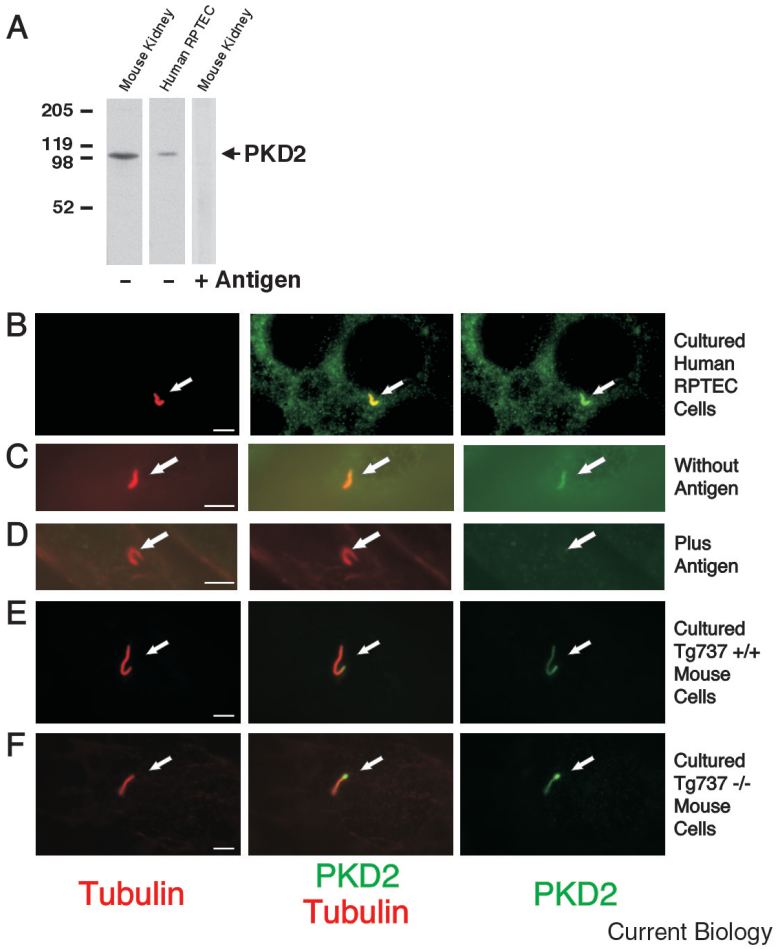

Figure 1. Subcellular localization of polycystin-2 in cultured kidney cells.
(A) Western blots of proteins extracted from mouse kidney and cultured human RPTEC cells. Blots were probed with a 1:5000 dilution of YCC2 anti-polycystin-2 sera. Bacterially expressed polycystin-2 antigen was added to the blot on the right as a control to show that the antibodies are recognizing polycystin-2 (PKD2). (B) Immunofluorescence images of human RPTEC cells labeled with antibodies to polyglutamylated ciliary tubulin (red, left panel) and polycystin-2 (PKD2) (green, right panel). Both are composite images made by combining one image taken in the plane of the cell body to show labeling of intracellular membranes and another image taken above the surface of the cells in the plane of the cilia. Colocalization of ciliary tubulin and polycystin-2 in the center panels is shown in orange over the cilia (arrows). Intracellular membranes are labeled only with the polycystin-2 antibody while the cilia are labeled with both antibodies. (C,D) Specificity of staining. RPTEC cells were labeled with antibodies to acetylated ciliary tubulin (red, left panels) and polycystin-2 (green, right panels) in the absence (C) and presence (D) of bacterially expressed polycystin-2 antigen. The bacterially expressed antigen blocked the ciliary and most of the internal membrane labeling by the polycystin-2 antibody but did not block labeling by the ciliary tubulin antibody. (E,F) Labeling of mouse kidney cells. Primary cells were isolated from kidneys of wild type (E) and *Tg737^{orpk}* (F) littermates and labeled with the anti-acetylated tubulin antibody (red, left panels) and the anti-polycystin-2 antibody (green, right panels). Size bars are 3 μm. Cilia are marked with arrows.

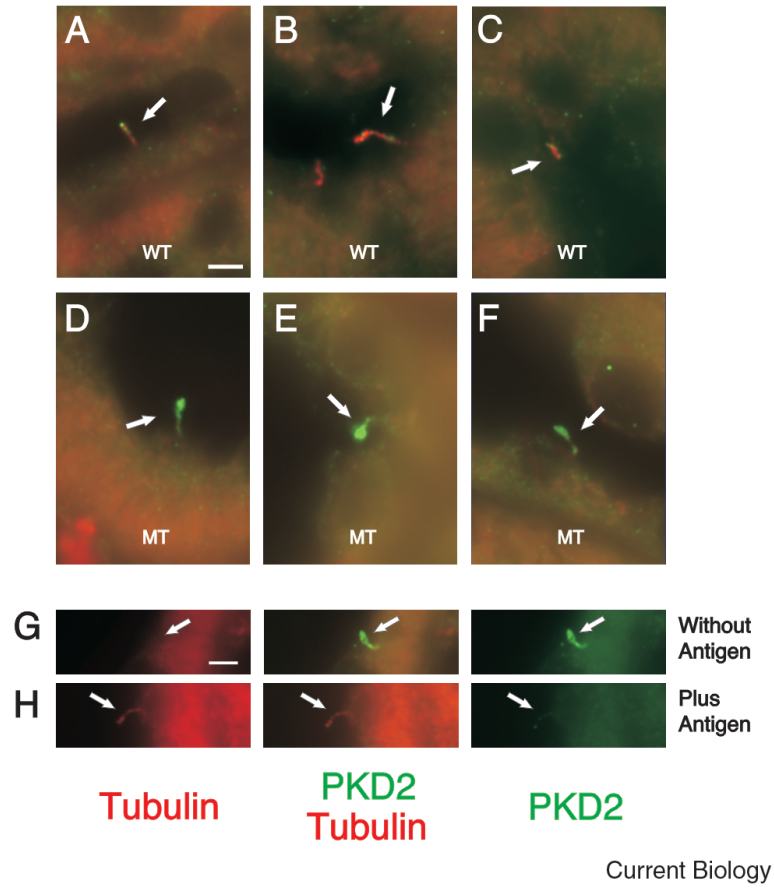

Figure 2. Subcellular localization of polycystin-2 in mouse kidney.
Wild type (A–C) and *Tg737*^{orpk} homozygous (D–F) mouse kidney sections labeled with the anti-acetylated tubulin antibody (red) and the anti-polycystin-2 antibody (green); red and green channels are merged in these images. Wild type cilia are weakly labeled with the polycystin-2 antibody but the mutant cilia are very strongly labeled; conversely, the mutant cilia are very weakly labeled by the anti-acetylated tubulin antibody, whereas the wild type cilia are strongly labeled. The cilia in (A,B,C,F) are in proximal tubules while those in (D,E) are in cortical cysts. (G,H) Specificity of staining. Histological sections of *Tg737*^{orpk} mutant mouse kidney were labeled with antibodies to acetylated ciliary tubulin (red, left panels) and polycystin-2 (green, right panels) in the absence (G) and presence (H) of bacterially expressed polycystin-2 antigen. The bacterially expressed antigen blocked the ciliary polycystin-2 labeling but did not block the labeling with the ciliary tubulin antibody. Cilia are marked with arrows. Size bars are 3 μm.

showing that the antibody is specific.

Labeling with antibodies to polycystin-2 (Figure 1B–F, right panels) and ciliary tubulin (Figure 1B–F, left panels) shows that in human (Figure 1B, C) and mouse (Figure 1E) kidney cells, polycystin-2 is concentrated in the cilia as well as present in the cell body. Polycystin-2 was seen in 87% of the RPTEC cilia (n=50) and 99% of the cultured mouse kidney cell cilia (n=100). Preincubation of the polycystin-2 antibody with antigen blocks the labeling of both the cilia and cell body (Figure 1C, D). The intracellular labeling presumably corresponds to the previously described endoplasmic reticulum pool of polycystin-2. It has been suggested that the endoplasmic reticulum is the site of polycystin-2 activity [7,8]; however, this pool may represent protein in transit to the ciliary membrane. Plasma membrane proteins such as the T-cell receptor and cystic fibrosis transmembrane conductance regulator are also abundant in the endomembrane system but act at the cell surface [9,10].

Tg737^{orpk} mutant mice have defects in assembly of kidney primary cilia and develop cystic kidneys [4,5]. To determine if the *Tg737*^{orpk} mutation affects the localization of polycystin-2, we examined cells derived from the kidneys of wild type and mutant animals as well as histological sections of wild type and mutant kidneys. Compared to those from a wild type mouse (Figure 1E), cilia on cells derived from the mutant animal are typically shorter and show stronger polycystin-2 labeling (Figure 1F), often associated with a bulge at the distal tip. The difference in staining is even more pronounced in histological sections. Cilia from wild type animals are weakly labeled with polycystin-2 antibodies (Figure 2A-C) but those from the mutant animals are strongly labeled in both cystic (Figure 2D, E) and non-cystic (Figure 2F) portions of the kidney. The elevated labeling was seen in 92% (n=500; from 4 animals) of the cilia from *Tg737*^{orpk} homozygous mutant animals but in fewer than 1% (n=500; from 4 animals) of the cilia of wild type and heterozygous animals. These results suggest that the *Tg737* gene product, IFT88, and intraflagellar transport are not required for moving polycystin-2 into cilia but may be needed to recycle it to the cell body.

Thus polycystin-2 is localized to kidney primary cilia and the primary cilium is likely to be an important site of polycystin action. These results imply a close mechanistic connection between PKD caused by defects in the polycystins, and PKD caused by defects in the primary cilia of the *orpk* mouse. Although primary cilia are extremely widespread in the adult mammal and are found on nearly all non-dividing cells [11], their function is unclear [12]. The current findings show that the kidney primary cilium is a sensory organelle displaying receptors that initiate a signal transduction pathway controlling cell differentiation and proliferation.

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Primer

Inner space: Reference frames

Aaron Batista

How are we aware of our surroundings? Somehow, from a pair of tiny, two-dimensional pictures of the visual world – provided by our retinas – our brains are able to render the world as it is, accurately depicting the locations of both a distant mountain range and the camera we are pointing at it. To understand how space is represented in the brain, neurophysiologists have borrowed the useful concept of a *reference frame* (also called a *coordinate frame*) from engineers and physicists. A reference frame, in the mathematical sense, is simply a set of rigid axes that intersect at a point, the origin. These axes are usually perpendicular to each other, and they are marked with gradations. This system allows the location of any object to be described by a set of numbers, called coordinates – its position along each of the axes. How is this notion useful in neuroscience? It allows us to phrase questions about how the brain encodes space in very concrete terms. For example, suppose we wish to understand how it is that a person can catch a baseball. Using reference frames, we can state the question as, 'How does the brain translate the position of the ball from the coordinates of the retinas into its coordinates in a reference frame centered on the left hand?' (Figure 1)

Some modifications to the physicist's concept of a reference frame are needed to apply it in neuroscience. Neurons in the visual system do not report the coordinates of an object. Instead, neurons that encode visual space each represent a very restricted region of space. Cells will respond to stimuli located in one particular location, termed the *response field* of the neuron, or the *receptive field* in cases where the neuron's response is considered to be strictly sensory, and will not respond to the same object positioned somewhere else. (Often, a visual stimulus must have other features in order for a neuron to respond to it, such as a particular color, a direction in which it is moving, or even how the animal intends to respond to that stimulus.) Areas of the brain that represent visual space do so by using a population of neurons with response fields at different locations. Once we locate the response field for a neuron, we can ask in which reference frame that neuron encodes space using some very simple manipulations: by moving one part of the body at a time, we can explore whether the neuron's response field moves along with that body part. If it does, then we have reason to believe that the neuron encodes space in a reference frame anchored to that body part.

Reference frames for vision

The neural signal induced by light in the retina is carried through the thalamus to the primary visual cortex, also called V1 or *striate cortex*, for the pronounced stripe of myelinated input fibers visible in histological sections. Neurons found along this pathway all use a *retinal* reference frame: if the eyes move, the spot in the world to which these cells respond also moves, but it stays fixed with respect to the retina. Retinal coordinates are also the rule throughout the *extrastriate* visual areas, those areas just downstream from the striate cortex. This fact leads to a dramatic realization about the organization of the visual system: every time our eyes move, the visual scene sweeps across our visual areas. Yet, despite this, we somehow perceive that the world remains stable.

To achieve this stability, the brain must factor in information about the position and movements of the eyes. This combination of visual and postural information becomes evident further along in the visual system. For example, area VIP, a multisensory area in the parietal lobe of monkeys, contains a